classical value of −1, but closer to the sol–gel transition a higher value is obtained (−1.7 ± 0.2), in agreement with the percolation theory and also with observations of other authors. The percolation-like behavior is of short duration (ca. $t_g/10$). Later in the reaction and in the subsequent gelations, such characteristic scattering features are probably masked by interference from neighboring clusters or from previous generations of gel.

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Registry No. (Acrylamide)(bisacrylamide) (copolymer), 25034-58-6.

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Interaction of Anionic Surfactants with Gelatin: Viscosity Effects

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ABSTRACT: Interactions of anionic surfactants with an alkali-processed gelatin above its isoelectric point were studied over a wide range of conditions. Extraordinarily large increases in viscosity were observed for some systems above a critical surfactant concentration, which nearly coincided with the critical micelle concentration for the corresponding gelatin–surfactant mixture. The general thickening effect is characterized by four distinct regimes corresponding to the level of surfactant in the gelatin solution. The extent of thickening was closely related to surfactant type, surfactant/gelatin composition, and ionic strength, with lesser effects exerted by gelatin type and pH level. The specific architecture of the surfactant molecule, particularly the size of the aliphatic moiety, had a decisive effect on the critical concentration for the onset of thickening and the ultimate thickening extent. A simple model, based on a cooperative micellar binding mechanism, is proposed to explain some of these observations.

Introduction

Interactions between surfactant molecules and synthetic or natural polymers have been studied extensively over the past several decades. These interactions have received considerable attention because of their ability to impart significant changes to the interfacial, rheological, and physicochemical properties of polymer systems, with important implications in various pharmaceutical, biomedical, food processing, and photographic applications.[1] Surfactant–polymer interactions involve various modes of association facilitated by dipole–dipole, ion–dipole or ion–ion forces. Nagarajan and Kalpakci[2] discuss six possible types of associations involving either individual surfactant molecules or surfactant clusters (micelles). In studies on mixtures of nonionic polymers with anionic surfactants,[1-3] a systematic drop in the critical micelle concentration (cmc) and moderate increases in viscosity have been observed. Evidence from nuclear magnetic resonance (NMR)[4] and neutron scattering[5] studies suggests the existence of polymer–micelle complexes in such systems. When both the surfactant and the polymer are charged, the interactions are dominated by strong Coulombic forces. Generally, the interaction of a surfactant with an oppositely charged polyelectrolyte results in precipitation.[6,7] Solubility of the polymer is, however, still possible at low concentrations of surfactant where complexation is not extensive and at very high concentrations where the complex is solubilized by excess surfactant. Large changes in viscosity at both concentration regimes have been observed.[8] Because of the unique electrolytic character of proteins, phenomena involving protein–surfactant interactions are especially intriguing. The ability of surfactants to denature and precipitate globular proteins and the disinfecting action of cationic detergents on bacteria are well-known.[9,10] Because of the diversity of polypeptide structures, it is not possible to generalize the consequences of interactions of proteins with surfactants. However, for denatured proteins one can distinguish two general cases:[10] (a) mixtures of anionic surfactants with proteins above the isoelectric point (IEP) (below the IEP for cationic surfactants) and (b) mixtures of anionic surfactants with proteins below the IEP (above the IEP for cationic surfactants). Since the protein has a net positive charge below the IEP and can be considered a "cationic" polymer, the interactions with anionic surfactants are dominated by precipitation phenomena. Above the IEP, the interactions lead to formation of stable, fully solubilized complexes which can lead to drastic changes in the topology and conformation of the protein molecule in solution.

In this study, we set out to explore the interaction of gelatin, a well-characterized denatured protein, with anionic surfactants, covering a wide range of compositions and conditions. As our main tool we used viscometry, which is convenient and particularly effective in probing

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conformational and topological changes in macromolecular systems. Associations that do not lead to systematic changes in the global configuration of the gelatin molecule cannot be studied by this method and are not considered here. This study is also limited to effects above the IEP.

Some of the earliest studies on gelatin–surfactant interactions are those by Pankhurst,[11] Kragh,[12] and Knox and co-workers.[13,14] In the studies of Kragh and Knox and Parshall, interactions above the IEP were investigated with surface tension techniques, whereas Knox and Wright studied precipitation phenomena in a gelatin–sodium dodecyl sulfate (S12S) system below the IEP using turbidimetric methods. Some changes in the viscosity of gelatin induced by the addition of S12S below and above the IEP were reported by Malik and co-workers.[15,16] Below the IEP, they observed a drop in viscosity for low surfactant levels and a moderate increase at high surfactant concentrations above the precipitation range. At and above the IEP, a systematic rise in viscosity with the addition of surfactant was observed. The viscometric effects in these studies were limited to a narrow range of gelatin concentration (<%1.5). Large thickening effects with the addition of polyanions and color couplers have also been reported. Uhrich and Nawn[17] describe the effect of several synthetic polyanions on gelatin viscosity and conclude that the ensuing interactions are predominantly electrostatic, involving the amino and guanidino groups on the polypeptide chain. In the study of Brand et al.,[18] a homologous series of color couplers with surfactant-like structures were investigated. The large rise in viscosity observed for these systems was attributed to the uncoiling and extension of the gelatin chain, due to increase in the net negative charge and the formation of a "sandwich-type micellar complex" consisting of two gelatin molecules bound by ordered pairs of surfactant molecules which are connected by hydrophobic bonds. More recently, Tavernier[19] studied viscometric and pH effects in gelatin mixtures with an anionic surfactant, *N*-methyl-*N*-oleoyltaurine. He attributes the observed rise in viscosity above the IEP to hydrophobic bridging of two gelatin molecules by pairs of surfactant molecules.

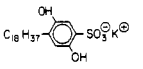
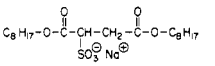
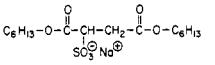
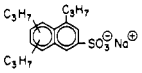
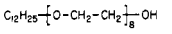
Experimental Section

Materials. (a) Gelatin. An alkali-processed deionized bone gelatin was used throughout this study. This gelatin has an IEP of 4.8 (determined viscometrically) and is buffered to pH 5.8. Unless otherwise noted, all runs in this study were carried out at this pH (±0.1). Gelatin solutions were prepared by soaking the gelatin flakes in distilled water for ~1 h, followed by heating to 41 °C with mild stirring. Gelatin concentrations quoted here are in weight percent of gelatin as supplied. The moisture content of the raw gelatin was determined to be 6.9 wt %.

(b) Surfactants. Most of the surfactants were reagent grade and used as received (Table I). By comparing data for a highly purified S12S grade (BDH Chemicals Ltd.) with data for a reagent grade, we could not detect significant differences in the viscometric response of the gelatin-S12S system (cf. Figure 2). In preparing the gelatin–surfactant solutions, the surfactants were dissolved in gelatin stock solutions at 40 °C.

Methods. (a) Viscometry. Viscosity was measured on a Low Shear 30 Contraves viscometer, which is a high-precision, small-volume Couette device. The shear rates covered a range of 0.01–100 s^{-1}, which is well within the Newtonian (linear) regime for all the solutions studied. All the viscometric measurements were run at 41 ± 0.1 °C. At this temperature, gelatin is denatured and can be represented as a random coil.[20] Intrinsic viscosities of S12S-doped gelatin solutions were measured with a Ubbelhode glass tube viscometer following a standard procedure. In order to avoid changes in the concentration of S12S during successive dilutions in the intrinsic runs, the stock solvents were aqueous solutions of S12S at a specified concentration. Also, in all the intrinsic viscosity runs, 0.1 M NaCl was added to the solution.

Table I
List of Surfactants

surfactant (designation)	source	structure[a]
sodium dodecyl sulfate (S12S)	Kodak	$C_{12}H_{25}SO_4^-Na^+$
sodium octyl sulfate (S8S)	Kodak	$C_8H_{17}SO_4^-Na^+$
sodium decyl sulfate (S10S)	Kodak	$C_{10}H_{21}SO_4^-Na^+$
sodium tetradecyl sulfate (S14S)	Kodak	$C_{14}H_{29}SO_4^-Na^+$
potassium octadecylhydroquinonesulfonate (P18HS)	Kodak	
Aerosol OT (AOT)	American Cyanamid	
Aerosol MA (AMA)	American Cyanamid	
Alkanol XC (AXC)	Du Pont	
Trycol LAL-8 (TL8)	Emery	

[a] As specified by vendor.

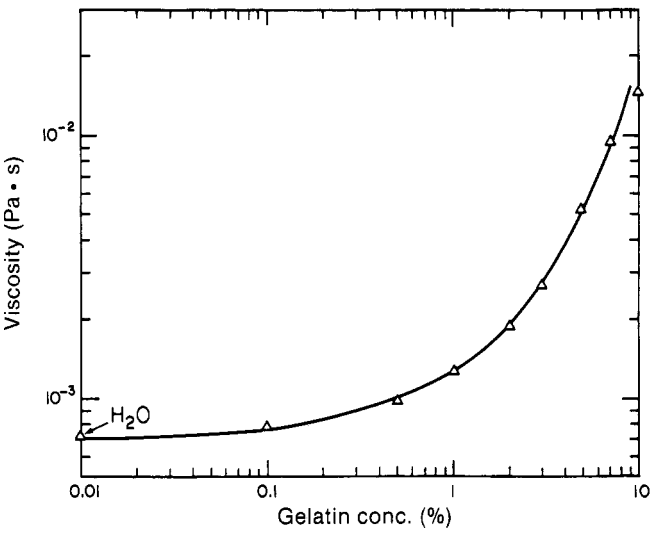


Figure 1. Viscosity of undoped aqueous gelatin solutions vs gelatin concentration (41 °C).

To probe the shear-thinning character of several gelatin–surfactant systems, some viscosity measurements were conducted at high shear rates (<50 000 s^{-1}). These measurements were run on a high-precision parallel-plate device (an accessory of the Rheometrics System Four rheometer) based on a recently developed procedure.[21]

(b) Cmc Determination by Fluorescence Probe. Gelatin solutions were prepared by using water previously saturated with 1-pyrenecarboxaldehyde. Solid S12S was added to the desired concentration. Fluorescence spectra were obtained with an SLM 4800S spectrofluorometer by excitation at 380 nm. Emission values were averaged from 10 points at each wavelength. The spectra were corrected for intrinsic fluorescence of gelatin by subtraction of spectra obtained with identically prepared samples without the fluorescent probe.

Results

The zero-shear-rate viscosity of aqueous gelatin solutions in the absence of surfactants is plotted in Figure 1 vs. gelatin concentration. A typical thickening curve for a gelatin–S12S system is shown in Figure 2. This curve traces the relative viscosity of the surfactant-doped gelatin solution vs. surfactant concentration, C_S, where the relative

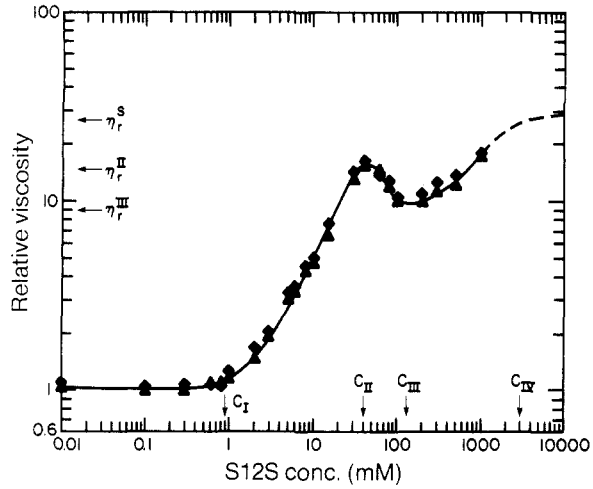


Figure 2. η_r vs S12S concentration for a 5% gelatin solution. Data for the reagent grade surfactant used in this study (▲) are compared with data for a highly purified grade (◆). The dashed curve is a plausible extrapolation of the data.

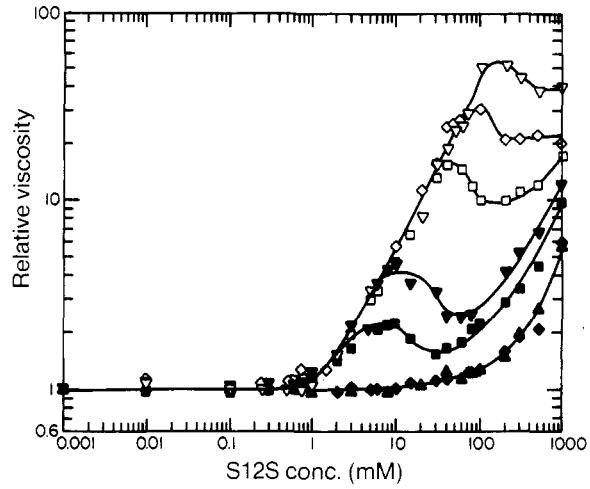


Figure 3. η_r vs S12S concentration. Effect of gelatin concentration: (▲) no gel; (◆) 0.1%; (■) 2.0%; (▼) 3.0%; (□) 5.0%; (◇) 7.0%; (▽) 10%.

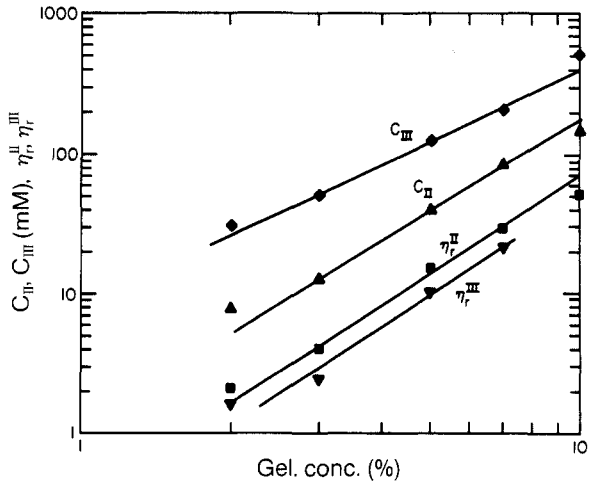


Figure 4. Thickening characteristics of gelatin–S12S mixtures vs gelatin concentration (see Figures 2 and 3).

viscosity is defined as the ratio of the viscosity of the surfactant-doped solution to that of the surfactant-free solution. The large changes in viscosity over the concentration range studied underlie the strong molecular interactions in this system and their dependence on the level of S12S. Three characteristic concentrations are identified in Figure 2: C_I, the threshold concentration, marks the onset of thickening; C_{II} corresponds to a local maximum in η_r (η_r^{II}); C_{III} is a local minimum (η_r^{III}). Accordingly, we have defined four regimes corresponding to these concentrations: nucleation ($C_S < C_I$); growth I ($C_I < C_S < C_{II}$); collapse ($C_{II} < C_S < C_{III}$); growth II ($C_S > C_{III}$). It is conceivable that the thickening curve will level off at some high concentration, $C_S = C_{IV}$, so that a fifth regime, saturation ($C_S > C_{IV}$), could be defined. However, saturation was observed only at the highest gelatin concentrations (7 and 10%) and the information on C_{IV} is presently scant. In the nucleation regime, the viscosity is unaffected by the presence of surfactant thus indicating that interactions, if present, do not induce significant conformational changes in the gelatin molecule. In the growth I regime, the relative viscosity rises nearly linearly with C_S, followed by a partial drop in viscosity in the third regime. Finally, in the fourth regime, growth II, the viscosity rises again, but at a different rate than in growth I.

The effect of gelatin concentration of the thickening response is shown in Figure 3. These data span a wide range of concentrations, going from a semidilute solution (0.1%) with a weak coil overlap to highly concentrated solutions. For comparison, the relative viscosity of the surfactant solution without gelatin (0%) is also shown in Figure 3. Here, the relative viscosity is the ratio of viscosities of the surfactant solution and water at 41 °C.

The effect of gelatin concentration, C_G, on the thickening characteristics defined in Figure 2 is depicted in Figure 4. For the concentrated solutions (2–10%), the threshold concentration, C_I, appears to be independent of C_G and the growth I regimes are congruent until the thickening curves diverge at C_{II}. However, as shown in Figure 4, C_{II}, C_{III}, η_r^{II}, and η_r^{III} are all strongly dependent on C_G. Thus, while the onset of thickening is independent of C_G, the ultimate and relative extents of thickening are intimately related to the gelatin/S12S composition. It is also noted that the thickening curve levels off (saturates) only for the two most concentrated solutions. For the lower concentrations, saturation is not reached within the range of the data. For the semidilute solution, 0.1%, the apparent C_I is shifted to a high C_S (20–40 mM) and the thickening curve closely follows the viscosity of a gelatin-free S12S solution. The thickening of the pure S12S solution is a result of the increase in the volume fraction of micelles (ϕ) with C_S, and it follows from the generalized Einstein equation[22]

$$\eta_r = 1 + 2.5\phi + 0(\phi^2) \quad (1)$$

We recall that the cmc for a pure S12S system is 8.5 mM,[23] which is somewhat below the apparent C_I for this system. The overlap in the viscosity curves of the 0.1 and 0% solutions implies that the thickening of the 0.1% solution, where S12S is much in excess to gelatin, is dominated by an increase in the volume fraction of S12S micelles, rather than by S12S–gelatin interactions.

As we further reduce the concentration of gelatin and digress into the dilute solution regime where molecular coils are not interacting, a distinctly different response is observed. Figure 5 shows the effect of S12S concentration on the intrinsic viscosity of gelatin. In this low concentration limit, the intrinsic viscosity drops with the addition of S12S, thus suggesting that the interaction of an isolated gelatin molecule with S12S leads to a partial collapse of the molecular coil. This effect is also operative at $C_S \gtrsim C_I$, where C_I has nearly the same value as before (Figure 3), but it is clearly in variance with the observations for the concentrated solutions. It follows then that the modes of gelatin–S12S association in the dilute and concentrated

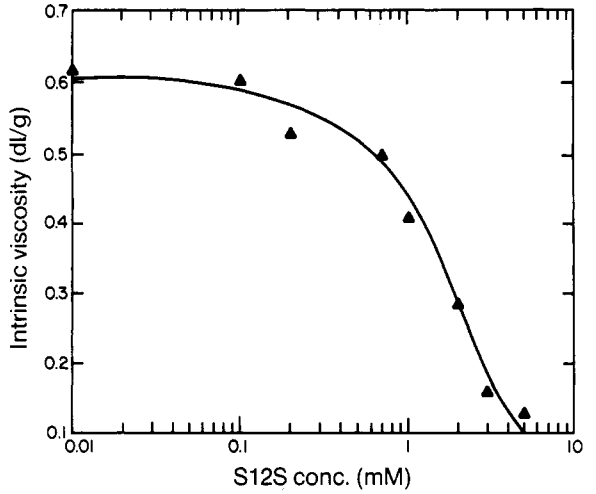


Figure 5. Intrinsic viscosity of gelatin (at 0.1 M NaCl, 40 °C) vs S12S concentration.

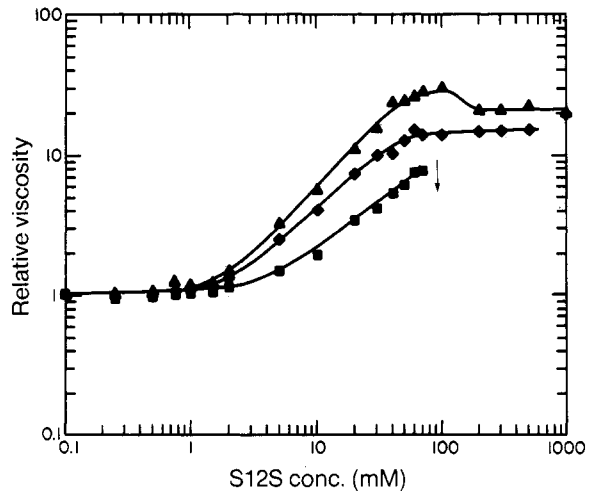


Figure 6. η_r vs S12S concentration for a 7% gelatin solution. Effect of ionic strength: (▲) no NaCl; (◆) 0.1 M NaCl; (■) 1.0 M NaCl. Arrow indicates precipitation.

regimes must be fundamentally different.

We proceed to examine the effect of the net charge on the gelatin molecule backbone and the role of electrostatic forces in the thickening response of gelatin. Thickening curves for three levels of ionic strength are shown in Figure 6. Clearly, the addition of salt suppresses the interaction to some extent as the level of thickening is reduced. However, even at exceedingly high levels of ionic strength (1 M NaCl), the effect cannot be completely suppressed and the rise in viscosity is still considerable. It is also seen that, although the shape of the thickening curve is altered by the addition of salt, C_I is practically unaffected. For the 0.1 M NaCl solution the "hump", corresponding to the η_r^{II} peak, virtually disappears and the viscosity levels off at a lower plateau. This could not be reproduced with the 1 M NaCl solution because of precipitation at $C_S > 80$ mM. The effect of pH on the viscosity of a particular gelatin–S12S system (selected from within the growth I regime) is shown in Figure 7. In this series, the pH was adjusted by potassium biphthalate (0.05 M) and potassium hydroxide–potassium carbonate–potassium borate (0.05 M) buffers keeping the concentrations of gelatin and S12S constant. At pH < 4.8 (IEP) precipitation occurred in line with the data of Knox and Wright.[13] Above the IEP, the viscosity passed through a maximum at pH 6.5. Below that point, coil expansion effects due to an increase in the net charge on the gelatin molecule may be partially responsible for the observed increase. Above pH 6.5, some of the

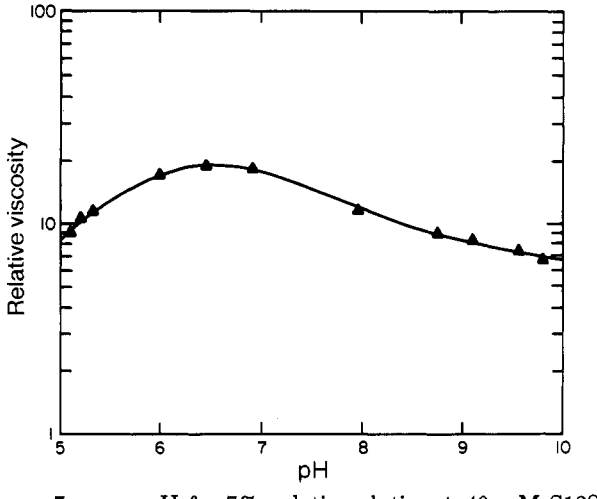


Figure 7. η_r vs pH for 7% gelatin solution + 40 mM S12S.

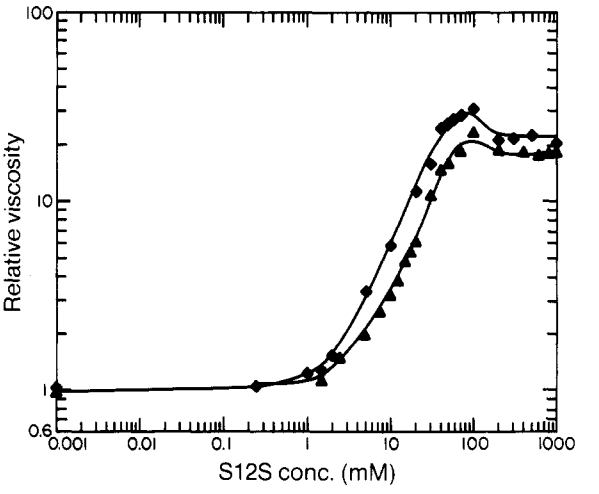


Figure 8. η_r vs S12S concentration for a 7% gelatin solution. Effect of gelatin type: (◆) deionized vs (▲) nondeionized.

positive charges on the polypeptide chain become neutralized (e.g., histidine has a pK_a of 6.5) while some are screened by free hydroxyl ions and, consequently, the binding with S12S is apparently suppressed.

A comparison between the standard deionized gelatin grade used here and a similar, but nondeionized, grade is shown in Figure 8. The difference in thickening extent between the two grades can be attributed to differences in charge distribution on the gelatin chain. An increased thickening of a deionized gelatin grade was also observed by Uhrich and Nawn[17] in their study of the interaction of polyanions with gelatin. The effects of ionic strength, pH, and gelatin type highlight the distinct electrostatic nature of the gelatin–S12S interactions. Evidently, these interactions can be partially suppressed by screening the active sites on the gelatin chain with free counterions.

The type and structure of the surfactant are also likely to play a major role in the interaction process, as already indicated by Knox and Parshall[14] and Brand et al.[18] In order to evaluate this effect, some of the experiments with S12S were repeated for several other surfactants. Figure 9 presents thickening curves for a 5% gelatin solution mixed with several sodium alkyl sulfates, with the alkyl group ranging from an octyl to a tetradecyl chain. The results clearly illustrate the great sensitivity of the thickening response of gelatin to structural changes in the surfactant. An increase in the size (and hydrophobicity) of the alkyl moiety shifts the onset of thickening (C_I) to lower concentrations and strongly enhances the level of thickening. This result is consistent with the findings of

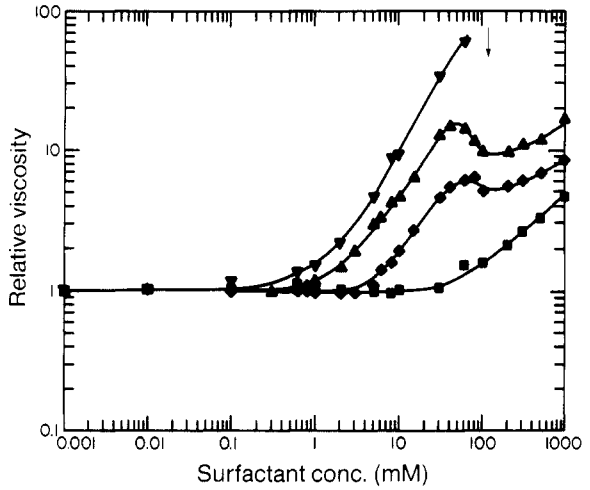


Figure 9. η_r vs surfactant concentration for a 5% gelatin solution. Effect of alkyl group in a series of sodium alkyl sulfates: (▲) S12S; (◆) S10S; (■) S8S; (▽) S14S. Arrow indicates precipitation.

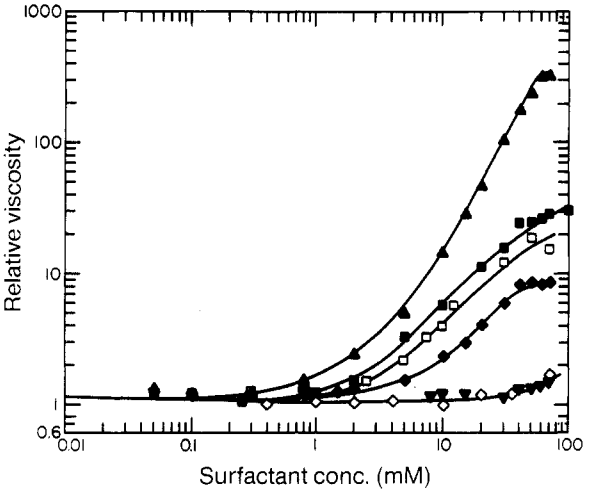


Figure 10. η_r vs surfactant concentration for 7% gelatin solution. Effect of surfactant type: (▲) P18HS; (◆) AXC; (■) S12S; (▼) AMA; (□) AOT; (◇) TL8.

Brand et al.,[18] who also observed a strong correlation between the size of the alkyl group (in the color couplers studied) and the increase in viscosity. These results also suggest that the concentration regimes defined earlier (Figure 2) are not specific to S12S; the overall thickening curve is reproduced closely for S10S, except that C_I, C_{II}, C_{III}, η_r^{II}, and η_r^{III} are markedly different. This implies that the interaction mechanisms for both surfactants are similar. This response could not be fully reproduced for S14S, because it could not be readily dissolved at $C_S > 60$ mM ($<C_{II}$). Similarly, because of a high threshhold concentration for S8S, the thickening curve for this surfactant is limited to only a portion of the growth I regime within the concentration range covered in this experiment. It is interesting to note that the slopes of the thickening curves in the growth I regime are similar for all the surfactants, except S8S. Similar slopes in the collapse and growth II regimes are observed for S10S and S12S. This suggests a similar association order for the surfactants in their binding with gelatin. The lower slope for S8S may indicate a lower association order for this surfactant. Data for a second group of surfactants are shown in Figure 10. Except for P18HS, all are commercial, widely used surfactants. The surfactants in this group are all anionic, except TL8. As in the previous group, the results in Figure 10 illustrate the great sensitivity of the thickening response of the system to even subtle structural changes in the

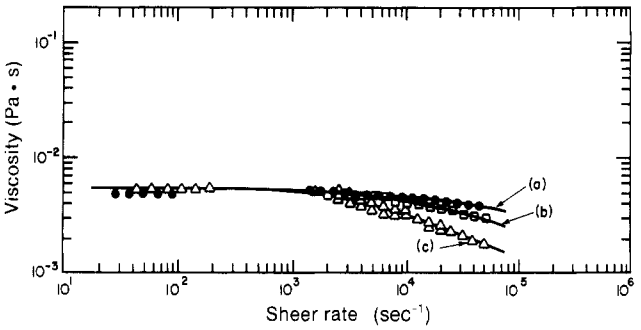


Figure 11. Flow curves for three gelatin–surfactant mixtures: (a) 15% gelatin; (b) 7% gelatin + 13 mM S12S; (c) 7% gelatin + 5.5 mM P18HS.

hydrophobic moiety of the surfactant molecule. For example, for $C_S = 70$ mM, the relative viscosity ranges from 1 (no thickening) for AMA and TL8 to ~350 for P18HS, implying a wide disparity in the degree of association among the various surfactants. Here too, the size of the alkyl group appears to be a decisive factor; two additional carbon units in the hydrophobic tail of AOT vis-à-vis AMA make it a fairly effective thickener compared to AMA, which has practically no effect on the viscosity of gelatin. Also, P18HS and S12S, with the largest alkyl groups in this series, are the most effective thickeners. Although the nonionic surfactant TL8 has the same hydrophobic moiety as S12S, it does not exhibit any thickening effect over the range of concentrations covered. Similar results (not shown) were also observed with Olin 10G (polyglycidol nonylphenyl ether), a nonionic surfactant. This further confirms that the gelatin–surfactant interactions are governed by Coulombic forces.

The viscometric behavior of gelatin at high shear rates should also provide important clues about the nature of its association with surfactants. Figure 11 presents flow curves for an undoped gelatin solution and two gelatin–surfactant systems, covering a wide range of shear rates. The high shear rates were attained by the procedure discussed in the Experimental Section. The three gelatin systems represent three levels of thickening, with the undoped gelatin being essentially unthickened ($\eta_r = 1$) and the P18HS–gelatin system having the highest η_r. These solutions were formulated so that their zero-shear-rate viscosities were the same, as shown in Figure 11. The data reveal distinct shear-thinning behavior for all three systems above sufficiently high shear rates. However, the differences in the onset and extent of shear thinning, in line with the level of thickening, suggest that association with surfactants leads to an apparent increase in the molecular size of the gelatin species (cross-linking?).[24] It is also noteworthy that no stress hysteresis (thixotropy) was observed in the high-shear experiments, thus implying that the formed complexes are sufficiently stable to withstand the strong hydrodynamic forces generated in this test.

We close this section by presenting some new data on the cmc of S12S in the presence of gelatin, which should be useful in elucidating the thickening mechanism to be discussed in the following section. The effect of gelatin on the cmc of S12S was studied in the past by using surface-tension techniques.[14] These measurements, however, were limited to low gelatin concentrations because of difficulties in reaching equilibrium for relatively viscous liquids. In this study, the cmc of S12S–gelatin solutions was determined by a fluorescence probe technique covering a wide range of gelatin concentrations corresponding to the viscometric measurements. Hydrophobic molecules that possess environmentally sensitive fluorescence prop-

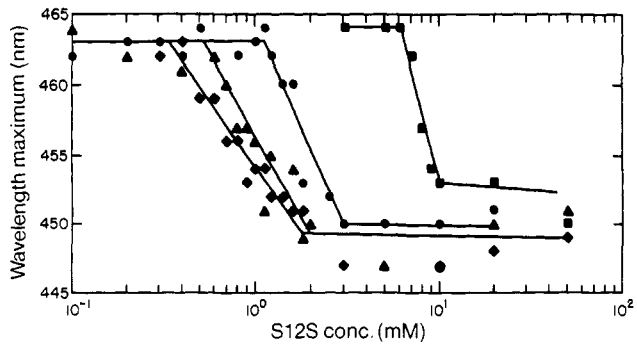


Figure 12. Fluorescence emission maximum for 1-pyrenecarboxaldehyde vs S12S concentration. Effect of gelatin concentration: (■) 0%; (●) 0.5%; (▲) 1.0%; (◆) 5.0%.

erties have been used in the past to study micelle formation and micelle properties of certain surfactants.[25-27] The probe used in this study was 1-pyrenecarboxaldehyde (1PC). The fluorescence maximum of the spectrum of this probe correlates well with the solvent polarity and can be related to the dielectric constant, D, of the medium by[25]

$$\lambda_{max} = 0.52D + 431.5 \quad (2)$$

where λ_{max} is the position of the peak maximum. Since 1PC is a very hydrophobic molecule, it is preferentially solubilized in or near the hydrophobic domain of the micelle, and the onset of micelle formation is reflected by a marked decrease in the wavelength of the peak emission. 1PC has been shown to be a reliable probe for cmc determination for several types of surfactants, and it does not noticeably affect the properties of the surfactant at the concentrations used in these measurements.[26] The influence of the S12S concentration on the emission maximum of 1PC is shown in Figure 12. In water, the probe possesses an emission maximum at 464 nm, while in the presence of S12S micelles in water, the emission maximum shifts to 453 nm. The change in emission maximum occurs abruptly at ~7 mM and it levels off again at 10 mM. If we consider the midpoint of the discontinuity in emission maximum to represent the effective cmc for this system, then we obtain a value of 8.5 mM which is identical with the well-known value of the cmc for S12S.[23] In the presence of gelatin, the cmc is reduced and it appears to be insensitive to gelatin concentration for relatively concentrated solutions (>1%). For the 0.5% solution a cmc value of 2 mM is found, while for the more concentrated solutions the cmc, based on the midpoint break in λ_{max}, is ~0.9 mM. This value is in close agreement with the results of Knox and Parshall[14] for a S12S + 1% gelatin solution.

Discussion

The viscometric data presented above underlie some important characteristics of the thickening response of gelatin in the presence of anionic surfactants. The salient features of this phenomenon are the following: (a) the onset of thickening (C_I) is surfactant specific and independent of gelatin concentration; (b) the extent of thickening is a strong function of gelatin/surfactant composition and surfactant type and is particularly sensitive to the size of the alkyl moiety in the surfactant molecule; (c) the gelatin–surfactant interaction is governed by Coulombic forces between the anionic group of the surfactant and positive charges along the gelatin molecule backbone. These charges are most likely the protonated amino and guanidino groups in the arginine and lysine amino acids, which are fairly abundant in the polypeptide sequence of gelatin.[17,18,20]

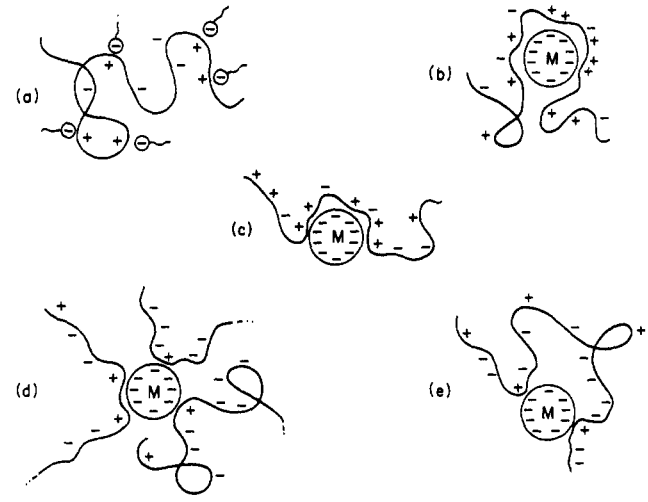


Figure 13. Possible association modes of denatured protein molecules with charged surfactants: (a) simple attachment of free surfactant; (b) micellar binding with complete shielding ("wrap around"); (c) micellar binding with partial shielding; (d) cooperative micellar binding ("cross-linking"); (e) intramolecular micellar binding.

The type and mode of interaction between the gelatin and surfactant molecules are key to our understanding of the thickening effect. Nagarajan and Kalpakci[2] discuss several types of polymer–surfactant complexes, some of which are reproduced in Figure 13. Only in one type (their type 6, see also Figure 13b) does the binding involve surfactant micelles associated with active sites on the polymer chain. They claim that an indirect clue to the formation of such "subaggregates" is provided by studies which show that the binding of some surfactants to nonionic polymers occurs above a critical concentration of surfactant. The existence of such a concentration implies that the binding is a cooperative process involving some aggregate of the surfactant molecules, possibly a micelle. Recent neutron scattering studies by Cabane and Duplessix[5] confirmed the existence of polymer–micelle complexes. Although most of these studies involved anionic surfactant–nonionic polymer systems, these general observations can be extended to charged polymers. The fact that the S12S cmc in the presence of gelatin, as obtained from fluorescence probe (Figure 12) and surface tension measurements,[14] is nearly coincident with the threshold concentration C_I (Figure 3) makes it plausible to assume that micelle formation is a key step in the binding process leading to thickening. Also, although we lack data on the cmc in the presence of gelatin of some of the other surfactants studied, the variation in C_I for these surfactants scales like the cmc for the undoped surfactants. Below C_I the free S12S molecules may bind to gelatin, but since the viscosity is unchanged this has apparently little effect on the global configuration of the gelatin molecule.

Once micelles are formed, a radical change in the topology of gelatin must ensue to explain the observed thickening response in the growth I regime. Several possible types of micelle–protein associations are shown schematically in Figure 13. Thus, the micelle can either bind intramolecularly, i.e., within a gelatin molecule, or it can bind cooperatively two or more molecules to form a large complex. The large levels of thickening observed and the enhancement of the shear-thinning character of the gelatin solution (Figures 3 and 11) for some of the systems studied cannot be explained merely in terms of a conformational change (unfolding) in a single gelatin chain but must involve some sort of cooperative association of several gelatin molecules leading, in effect, to an ap-

parent increase in molecular weight. This argument favors the "cross-linking" association in Figure 13d. The intramolecular binding is expected to collapse the gelatin coil, and thereby reduce its ability to dissipate energy, leading to a drop in viscosity. Indeed, in the limit of the dilute solution regime where the macromolecular coils are sufficiently apart, this is the only possible mode of interaction and the observed drop in intrinsic viscosity, shown in Figure 5, seems to bear this out. The binding of several molecules by a single micelle is a highly cooperative process and is possible only for concentrated solutions of gelatin molecules with a sufficient number of binding sites which are readily accessible to the negatively charged micelle. Clearly, the "wrap around" model (Figure 13b) proposed by Nagarajan and Kalpakci[2] for a poly(ethylene oxide)–S12S system cannot be operative in this case, since it will result in shielding of the micelle and prevent it from interacting with additional gelatin molecules. Also, since above the IEP the gelatin molecule is predominantly negatively charged, the microenvironment in the vicinity of the binding site is likely to have a net negative charge. Thus the peptide chain near the binding site will be repelled from the surface of the micelle, as shown in Figure 13d, leaving the micelle exposed and ready to associate with additional gelatin molecules. Below the IEP, a complete shielding (see Figure 13b) is expected, leading ultimately to precipitation. However, at a pH range slightly above the IEP, partial shielding (Figure 13c) may occur, thus reducing the ability of the micelle to bind with free gelatin strands. This may explain the increase in thickening with pH observed for a pH range 4.8–6.5 (cf. Figure 7). At higher pH levels, the drop in thickening with increase in pH is attributed to screening and neutralization of the binding (positive) sites on the gelatin molecule backbone.

The number of molecules that can be bound by a single micelle ("functionality") should be a direct measure of its size, as well as the degree of shielding. Large micelles should have a higher functionality leading to larger complexes and, consequently, higher levels of thickening. Indeed, the large disparity in the thickening capacities of the anionic surfactants studied (Figures 9 and 10) can be attributed to differences in the aggregation number of the corresponding micelles. This is consistent with the observation that surfactants with the largest alkyl groups, e.g., S14S and P18HS, are also the most effective thickeners.

The sharp rise in viscosity with increase in the size of the complex is due to the strong molecular weight dependence of the zero-shear-rate viscosity. In fact, for linear uncharged polymer systems with molecular weights above the entanglement molecular weight, the viscosity is scaled by[24]

$$\eta \propto M^{3.4} \tag{3}$$

If the change in molecular weight involves also a change in topology, as we presume to be the case here, then eq 3 may not be strictly valid although the molecular weight dependence of the viscosity is expected to remain strong. Indeed, in recent studies on star molecules,[28-30] which are somewhat reminiscent of the gelatin–surfactant complexes as represented by Figure 13d, it was reported that the molecular weight dependence of viscosity is exponential(!). Since the proposed topology and general physicochemical character of our system are distinct from those studied in the literature, we replace eq 3 with a generalized power law

$$\eta \propto M^{\alpha} \tag{3a}$$

with the understanding that the exponent α is much greater than unity and, in all liklihood, even greater than 3.4. Thus, if n gelatin molecules are bound per micelle, the viscosity is expected to rise by a factor of n^{α}.[31] This, of course, would be the case only if the complexation reaction goes to completion, i.e., if all the unbound gelatin chains transform fully into n gelatin complexes. The formation of these complexes occurs in the growth I regime. During this phase, the viscosity of the solution can be expressed by

$$\eta = f(\phi_i,\eta_i) \qquad i = 1, ..., n \tag{4}$$

where ϕ_i and η_i are the volume fraction and viscosity of a complex which is comprised of i gelatin strands and f is some unspecified function. As surfactant is added to solution, the volume fraction of the higher order complexes is increased, while the unbound gelatin molecules are gradually depleted. If we assume linear additivity[32] and neglect the first-order term due to the strong dependence of η_i on i,

$$\eta_r \sim \sum_{i=2}^{n} \phi_i \eta_i \tag{5}$$

If only a single micelle participates in the formation of a G_i·M complex, then $\phi_i \sim C_S$, where M represents the micelle, G is the gelatin strand, and C_S is the surfactant concentration ($>C_I$), and we can write, in general, for the growth I regime

$$\eta_r \sim C_S \sum_{i=2}^{n} k_i i^{\alpha} \tag{6}$$

where k_i are characteristic constants of the G_i·M complexes. Only at saturation we get

$$\eta_r \sim n^{\alpha} \tag{7}$$

However, because of the highly cooperative nature of the binding process, saturation can be achieved only with a large excess of binding sites, i.e., high gelatin concentration C_G with no screening. If accessible binding sites are depleted (e.g., this will be the case for low C_G), then thickening will cease with the addition of surfactant before saturation is reached. This event is marked by C_{II} in our thickening scheme. Overall, the observed thickening response in the growth I regime is consistent with this qualitative picture. The proportionality of η_r to C_S in the growth I regime (slope of 1 in a log–log space) implies that a single micelle per complex is involved in the binding process (cf. eq 6). Also, saturation is observed only for the 7 and 10% solutions with a η_r^{II} in the range 30–50. For $\alpha = 3.4$, this may suggest a functionality of $\sim$3 (cf. eq 7) for S12S or a contact area of $\sim$1700 Å^2 per gelatin molecule out of a total surface area of $\sim$5000 Å^2 for a typical S12S micelle. The fact that C_{II} increases with C_G indicates that accessible binding sites are depleted at higher C_S when the number of gelatin molecules is increased. If C_G is not sufficiently high, the complexation process will not be complete at C_{II}; i.e., some molecules will remain unbound and some complexes will contain a lower number of gelatin chains ($<n$). This is consistent with the strong dependence of C_{II} on C_G (Figure 4). It should be noted that depletion can also be induced by screening the gelatin with counterions, as illustrated in Figures 6 and 7.

At C_{II}, the third concentration regime commences with a consistent drop in viscosity at this stage ($\eta_r^{III}/\eta_r^{II} \sim {}^2/_3$). This may be caused by a partial collapse due to intramolecular (intracomplex) binding by newly formed micelles and also possibly due to conformational changes induced by screening with an excess of surfactant. Since the co-

operative binding is exhausted at C_{II}, the newly added micelles will penetrate the gelatin complex and bind to one or more sites within the complex, which will lead to a collapse in its dimensions and an eventual drop in viscosity. This effect is not unlike the drop in intrinsic viscosity observed for dilute gelatin solutions (Figure 5). In both cases, the only possible mode of micelle interaction with gelatin is intramolecular. Once the sites within the complex are depleted, the various species (G, G·M, G_2·M, ..., G_n·M) will start interacting to form yet larger complexes leading again to a rise in viscosity (growth II). The rate of viscosity rise at this phase is somewhat lower than in the growth I regime, possibly because of a lower number of available sites and lower mobility of the interacting species. Another possible mode of interaction which may lead to a rise in viscosity is the attachment of multiple micelles to the gelatin complexes.

The cooperative micellar association mechanism proposed above can be represented by the following set of dynamic equilibria:

I. Nucleation ($C_S < C_I$)

$$G + S \rightleftharpoons G\cdot S \quad (8)$$

$$mS \rightleftharpoons M \text{ (at cmc} \sim C_I) \quad (9)$$

II. Growth I ($C_I < C_S < C_{II}$)

$$G + S \rightleftharpoons G\cdot S \quad (10)$$

$$G + M \rightleftharpoons G\cdot M \quad (11)$$

$$G\cdot M + G \rightleftharpoons G_2\cdot M \quad (12)$$

$$\vdots$$

$$G_{n-1}\cdot M + G \rightleftharpoons G_n\cdot M \quad (13)$$

III. Collapse ($C_{II} < C_S < C_{III}$)

$$G_i\cdot M + M \rightleftharpoons M\cdot G_i\cdot M \quad (14)$$

IV. Growth II ($C_{III} < C_S < C_{IV}$)

$$G_i\cdot M_j + G_{i'}\cdot M_{j'} \rightleftharpoons M_{(j+j')}\cdot G_{(i+i')} \quad (15)$$

or

$$G_i\cdot M_j + kM \rightleftharpoons M_{(j+k)}\cdot G_i \quad (16)$$

Based on the preceding discussion, eq 9 is the rate-determining step in the nucleation phase where m, the aggregation number, as well as C_I is surfactant specific. In the growth I phase, the rate-determining steps are all the reactions involved in the cooperative binding process, viz., eq 12 and 13. If, for example, we assume a functionality of 3 for the gelatin–S12S system, as implied by the relative viscosity at saturation, then the cooperative binding reactions can be combined to

$$G\cdot M + 2G \rightleftharpoons G_3\cdot M \quad (17)$$

This reaction is second order in G, consistent with the C_G^2 dependence of C_{II} (Figure 4). In general, we expect C_{II} to scale with C_G^{n-1}, where n is surfactant specific and intimately related to m. The mechanistic details of the collapse and growth II phases are more difficult to infer from the available body of data, and the corresponding ideas should be considered highly speculative at this time. Finally, we note that the micellar cooperative binding mechanism proposed here is in variance with the hydrophobic bonding mechanism of Tavernier,[19] but it is fully consistent and more in line with the experimental data presented in this and other studies.

Summary and Conclusions

On the basis of the data presented, the interaction of anionic surfactants with denatured gelatin above its isoelectric point is characterized by several distinct features: (a) under some conditions, the interaction is manifested by a substantial rise in viscosity (thickening); (b) the thickening process can be divided into four distinct regimes, each representing a different mode of intermolecular association; (c) the onset of thickening is independent of gelatin concentration above sufficiently high C_G but is intimately related to the type and structure of the surfactant; (d) the extent of thickening is a function of the gel/surfactant composition and the structure of the surfactant; (e) the interaction is governed by electrostatic forces. The micellar binding mechanism proposed here is fully consistent with these observations; it explains the existence of a critical thickening concentration (C_I) and the extraordinarily large increases in viscosity observed for some systems, as well as the dependence of these effects on changes in surfactant structure and ionic strength. Also, based on the proposed mechanism, the overall shape of the thickening curve can be used to estimate the "functionality" of the micelle and the association order of the gelatin–micelle complex. Drops in viscosity observed in the third regime and for a dilute gelatin solution are explained in terms of an intramolecular (intracomplex) binding process which leads to a partial collapse of the molecular coil. Some of the subtler aspects of the thickening effect, especially the response in the third and fourth regimes, are more difficult to rationalize via the proposed mechanism, based on the available body of data. More complete resolution of these questions will require additional data at high concentrations of surfactant and gelatin.

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Registry No. S12S, 151-21-3; S8S, 142-31-4; S10S, 142-87-0; S14S, 1191-50-0; P18HS, 24730-07-4; AOT, 577-11-7; AMA, 6001-97-4; AXC, 65863-15-2; TL8, 9002-92-0; 1-pyrenecarboxaldehyde, 3029-19-4; tripropyl-2-naphthalenesulfonic acid sodium salt, 110095-05-1.

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Dissociation Behavior of an Alternating Copolymer of Isobutylene and Maleic Acid by Potentiometric Titration and Intrinsic Viscosity

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ABSTRACT: The potentiometric titration and the intrinsic viscosity of the alternating copolymer of isobutylene and maleic acid were measured to study its dissociation behavior under various conditions. The results of the potentiometric titration show that the carboxyl groups in the copolymer appear to dissociate in two steps without any abnormal behavior due to the hydrophobic effect of the two methyl groups in the isobutylene units over the entire range of the degree of dissociation. The intrinsic viscosity of this copolymer has a clear maximum nearly at a half of the degree of dissociation and a linear relationship to the reciprocal square root of the concentration of the added salt. The dissociation behavior of this alternating copolymer is discussed in terms of the short-range and the long-range electrostatic interaction of the dissociation groups by the use of a linear Ising model. It is found that the model calculation is in better agreement with the experimental results if either a lower dielectric constant or hydrogen bonding between the nearest-neighbor carboxylic groups is assumed.

Introduction

The solution properties of the alternating copolymers of maleic acid with the hydrophobic monomers such as alkyl vinyl ethers or styrene have been studied in great detail by Strauss et al.[1] and Sugai et al.[2] Their primary concerns were in most cases the interrelation between the hydrophilic and the hydrophobic effects on various kinds of solution properties of such copolymers. Some exhibit clear evidence of a conformational transition from a compact globule to an extended coil upon increase of the degree of dissociation, α, of the carboxyl group in the maleic acid units. According to the results obtained by Dubin and Strauss[3] on the alternating copolymers of maleic acid with alkyl vinyl ethers, such a conformational transition seems to become clearer as the number of the carbons in the alkyl substituent becomes larger; for example, the alternating copolymer of maleic acid with octyl vinyl ether has a clear conformational transition whereas that with ethyl vinyl ether does not.[4] Similar phenomena are also observed in a certain poly(carboxylic acid) having hydrophobic substituents such as poly(methacrylic acid), PMeA,[5] which carries a methyl group at the α-position of COOH. However, such a conformational transition of poly(carboxylic acid) homopolymers seems to be caused by a very subtle change in the conditions, since the homologous polyacid, poly(crotonic acid), PCA, having a methyl group at the β-position, does show little sign of such a conformational transition.[6] These observations may suggest that besides the size of the hydrophobic group its arrangement in a polymer chain may be an important factor for such a conformational transition.

From another point of view it is also interesting to elucidate why the dissociation of the carboxyl groups in the alternating copolymers of maleic acid appears in two steps, since these copolymers must have the same linear charge densities as those of the common vinylic poly(carboxylic

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